METHOD AND COMPOSITION FOR CONTROLLING ORAL PATHOGENS

STATEMENT OF GOVERNMENTAL INTEREST

FIELD OF THE INVENTION

10   The present invention relates to the con-
trol of oral and other human pathogens, and to the
treatment of diseases and conditions that directly
or indirectly result from such pathogens, by admin-
istering (a) berberine and (b) an antimicrobial or
15 an antibiotic. More particularly, the present
invention relates to a method of potentiating the
effects of an antimicrobial or an antibiotic in a
mammal by administration of therapeutically effec-
tive amounts of both berberine and an antimicrobial
20 or an antibiotic. The composition and method of the
present invention permit a reduction in the anti-
microbial or antibiotic dose and in the berberine
dose while providing a desired therapeutic effect,
thus reducing adverse side effects.

25   ### BACKGROUND OF THE INVENTION

Dental plaque frequently is associated
with oral diseases, including dental caries and
periodontal disease (i.e., gum disease). Plaque

control can be achieved by mechanical (e.g., brush-
ing and flossing) or chemical means. For example,
antimicrobial agents have been incorporated into
oral hygiene products, such as toothpastes and mouth
5   rinses, to control plaque and gingivitis/periodontal
disease.

At present, the antimicrobial chlorhex-
idine (CHX) is the "gold standard" of plaque control
agents and is included in several oral hygiene prod-
10   ucts. However, CHX has many adverse side effects,
including an undesirable taste, tooth discoloration,
and increased calculus (i.e., tartar) formation.

Tetracyclines are conventional antibi-
otics, and routinely are used in the treatment of
15   periodontal disease because of their unique ability
to accumulate in the periodontal pocket area. How-
ever, like other conventional antibiotics, many
bacteria, especially important human pathogens, have
developed a resistance to antibiotics. Tetracycline
20   antibiotics also have many adverse side effects,
including gastrointestinal discomfort, nausea,
vomiting, diarrhea, and permanent tooth discolora-
tion in children up to eight years old.

Berberine is a plant alkaloid having a
25   long history of medicinal use in both Ayurvedic and
Chinese medicine. Berberine is present in the
roots, rhizomes, and stem bark of *Hydrastis canaden-*
sis (goldenseal), *Coptis chinensis* (coptis or
goldenthread), *Berberis aquifolium* (Oregon grape),
30   *Berberis vulgaris* (barberry), and *Berberis aristata*
(tree tumeric). Berberine is 5,6-dihydro-9,10-

dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizin-
ium, and as used herein, the term "berberine"
includes free berberine and salts thereof.

Goldenseal is a major phytomedicine sold
5   in the United States and is one of the top five
herbal supplements in the worldwide market. Golden-
seal is used in various dietary supplements for the
purpose of enhancing the immune response of the
body. Goldenseal-containing oral hygiene products
10.   also are available over the counter, but limited
information exists with respect to the efficacy of
goldenseal in oral health applications.

Berberine extracts and decoctions demon-
strate significant antimicrobial activity against a
15   variety of organisms, including bacteria, viruses,
fungi, protosoans, helminthes, and chlamydia.
Currently, the predominant clinical uses of berber-
ine include treatment of bacterial diarrhea, intes-
tinal parasite infections, and ocular trachoma
20   infections.

As previously stated, antimicrobials and
antibiotics exhibit several adverse side effects,
including tooth discoloration, bad taste, and
nausea, but they are routinely used in the treatment
25   of periodontal disease. In accordance with the
present invention, a combination treatment utilizing
berberine and a conventional antimicrobial or anti-
biotic agent allows for decreased concentrations of
both agents, which reduces the occurrence or sever-
30   ity of adverse side effects. Such a finding is an
important advance in the art. The adverse side

effects attributed to antibiotics limit the useful-
ness of antimicrobials and antibiotics, like tetra-
cyclines, and antimicrobials, like CHX, in the
treatment and control of diseases and conditions of
5   the mouth, teeth, and gums. The present invention,
therefore, is directed to the discovery that an
antimicrobial or antibiotic and berberine, when
administered in combination, perform synergistical-
ly. Therefore, the amount of antimicrobial or anti-
10 biotic and berberine used to control the oral path-
ogens can be reduced, with a concomitant reduction
or elimination of adverse side effects. The com-
bined use of an antimicrobial or antibiotic and a
berberine also can be used to synergistically con-
15 trol nonoral human pathogens.

Additional background information can be
found in Appendix A, which constitutes a portion of
this disclosure.

SUMMARY OF THE INVENTION

20   In a programmed research effort, it was
found that administration of berberine potentiates
the antimicrobial effects of an antimicrobial or
antibiotic in the treatment of oral and other human
pathogens. Administration of an antimicrobial or
25 antibiotic (hereafter "antibiotic") in combination
with berberine synergistically potentiates the anti-
microbial effect of an antibiotic, and, therefore,
lowers the dose of both the antibiotic and berberine
required to provide a desired antimicrobial effect.
30 The reduced amount of antibiotic and berberine re-

quired to provide a desired antimicrobial effect
reduces the severity of various adverse side effects
associated with antibiotic and berberine treatment.

     Accordingly, one aspect of the present in-
5   vention is to provide a composition comprising (a)
an antibiotic, e.g., CHX, tetracycline, or doxy-
cycline, and (b) berberine, or a salt, derivative,
or prodrug thereof, for use in controlling oral and
other human pathogens.

10   The present invention, therefore, provides
a composition and method for improved control of
oral pathogens, and treatment of diseases and condi-
tions caused by oral pathogens, like gingivitis and
dental caries. In particular, the present invention
15   is directed to compositions containing berberine and
an antibiotic, and to methods of using the composi-
tion to prevent and/or treat diseases and conditions
of the mouth, teeth, and gums. More particularly,
the present invention is directed to compositions
20   containing berberine and CHX or a tetracycline, and
to use of the compositions in methods of treating
diseases and conditions of the mouth, teeth, and
gums.

     Another aspect of the present invention is
25   to provide a method and composition for controlling
oral pathogens, and for treating conditions and
diseases caused by such pathogens, while reducing
the adverse side effects associated with berberine
and antibiotic treatment.

30   Yet another aspect of the present inven-
tion is to provide a method and composition for con-

trolling nonoral human pathogens, and treating con-
ditions and diseases directly or indirectly caused
by such pathogens.

5   Still another aspect of the present inven-
tion is to provide a method and composition for con-
trolling oral and nonoral human pathogens, including
antibiotic-resistant pathogens.

Another aspect of the present invention is
to provide an article of manufacture for human use
10   comprising (a) a package insert, (b) a container,
and either (c1) a packaged composition comprising an
antibiotic and berberine or (c2) a packaged composi-
tion comprising an antibiotic and a packaged com-
position comprising berberine.

15   These and other aspects of the present
invention will become apparent from the following
detailed description of the preferred embodiments of
the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

20   The present invention also is described in
detail in the attached Appendix A, which constitutes
a portion of the present disclosure.

In particular, Appendix A discloses test
results relating to administration of a berberine
25   and an antibiotic to control oral pathogens. The
tests show that administration of berberine syner-
gistically potentiates the antimicrobial effect of
an antibiotic, and allows a reduction in the dose of
both the antibiotic and berberine, while providing
30   an antimicrobial effect equivalent to using a higher

dose of antibiotic or berberine used alone. Such a synergistic coadministration provides an effective treatment, reduces adverse side effects associated with antibiotic or berberine administration, and

5 addresses the problem of bacterial resistance to antibiotics.

The present invention, therefore, provides methods of synergistically potentiating the anti-microbial properties of an antibiotic and berberine

10 in the treatment of oral and other human pathogens. The invention also provides pharmaceutical composi-tions comprising an antibiotic and berberine.

The present invention also provides arti-cles of manufacture comprising an antibiotic and

15 berberine, packaged separately or together, and an insert having instructions for using these active agents in the control of oral and other human path-ogens, and the treatment of diseases and conditions caused by such pathogens.

20 The methods described herein benefit from the use of an antibiotic and berberine in the treat-ment and management of oral and other human path-ogens. The antibiotic and berberine can be admin-istered simultaneously or sequentially to achieve

25 the desired synergistic antimicrobial effect.

For the purposes of the description here-in, including Appendix A, the term "treatment" includes preventing, controlling, lowering, or eliminating the concentration of oral and other

30 human pathogens. As such, the term "treatment"

includes both medical therapeutic and/or prophyl-
actic administration, as appropriate.

The term "container" means any receptacle
and optional closure therefor suitable for storing,
5   shipping, dispensing, and/or handling an article of
manufacture.

The term "insert" means information accom-
panying an article of manufacture that provides a
description of how to administer the article, along
10   with the safety and efficacy data required to allow
the physician, pharmacist, or user to make an in-
formed decision regarding use of the article. The
package insert generally is regarded as the "label"
for an article.

15   The term "prodrug" means compounds that
transform rapidly in vivo to a compound useful in
the invention, for example, by hydrolysis. A
thorough discussion of prodrugs is provided in
Higuchi et al., *Prodrugs as Novel Delivery Systems*,
20   *Vol. 14*, of the A.C.S.D. Symposium Series, and in
Roche (ed.) *Bioreversible Carriers in Drug Design*,
American Pharmaceutical Association and Pergamon
Press, 1987.

In accordance with an important feature of
25   the present invention, an antibiotic and a berberine
are present in a composition, or are administered in
combination, in a weight ratio of antibiotic-to-
berberine of about 0.01:1 to about 100:1, and pref-
erably about 0.01:1 to about 10:1. This ratio de-
30   pends upon the type and identity of antibiotic and
berberine being used. The ratio of antibiotic-to-

berberine that is administered is dependent upon the
particular antibiotic and berberine used, and the
origin and severity of the condition being treated.
This ratio can be readily determined by a person
5   skilled in the art to achieve the desired control of
an oral or other human pathogen.

When berberine is used in combination with
an antibiotic, such as CHX or a tetracycline, like
doxycycline, a synergistic antimicrobial growth
10   inhibition effect is observed. Such a combination
treatment results in a lower minimum inhibitory con-
centration (MIC) of either active agent.

Antibiotics most commonly are used in
dental practice as prophylactic agents for preventa-
15   tive management of endocarditis. In addition, anti-
biotics are used therapeutically in cases where
infections of oral hard and soft tissues, such as
teeth and gingival, cannot be controlled by local
debridement and can spread to distant organs, and,
20   therefore, require supplemental therapy.

Tetracycline antibiotics have a broad
spectrum of activity, can be used by many routes of
administration, such as oral administration and sus-
tained delivery systems, and are widely used.
25   Tetracyclines inhibit protein synthesis by combining
the small (30S) subunit of the ribosome and inhibit-
ing the binding of aminocyl-tRNA molecules to the
ribosomal A site. Antibiotics generally are con-
sidered bacteriostatic.

30   Reports of bacterial resistance to tetra-
cyclines, both *in vitro* and *in vivo*, are increasing.

However, antibiotics still are useful, and are rou-
tinely used in periodontal treatment. Tetracyclines
concentrate in periodontal pockets at inhibitory
levels 2 to 4 times higher than in the blood,
5   strongly bind to root surfaces, and can be released
in active form over extended time periods. Sub-
lethal concentrations of tetracyclines reduce
adherence and coaggregation properties of a number
of pathogens and also inhibit the collagenolytic
10   enzymes of mammalian cells enabling periodontal
ligament regeneration. But, if the pathogen is a
resistant to antibiotics, the periodontal disease
may not be treated, but rather may be aggravated.

Chlorhexidine (CHX) is a bisbiguanide
15   biocide, and is marketed in the form of different
salts. Chlorhexidine gluconate is one of the most
common of these salts. CHX-containing compositions
have been used as topical disinfectants since the
middle 1970s. Because chlorhexidine in the salt
20   form is cationic, it readily binds to negatively
charged cell walls. CHX targets the cytoplasmic
membrane of bacteria resulting in the loss of
structural organization and integrity. Subse-
quently, coagulation and precipitation of cyto-
25   plasmic constituents occurs.

In patients having high caries activity
and high counts of Streptococci mutans, CHX can be
employed as an adjunct to other preventative
measures. In periodontal treatment, chlorhexidine
30   is used for postoperative rinsings and as an adjunct
to mechanical debriment. CHX compositions also are

used for nonspecific plaque control, as well as
against specific bacteria associated with perio-
dontal disease and caries and in subgingival irriga-
tion. CHX usually is administered from rinsing
5   solutions (concentrations, typically about 1-2%),
and slow release devices, such as varnishes, fibers,
and slab-like sustained delivery devices. CHX also
can be incorporated into chewing gum, toothpastes,
and toothbrushes, or in an oral rinse or in a disin-
10   fectant for dentures in patients with candidial in-
fections. CHX has a low level of toxicity both
locally and systemically and shows no permanent
retention in the body.

The antibiotic incorporated into a present
15   composition or utilized in a present method is not
limited to CHX. Any other antibiotic useful in the
treatment of oral and other human pathogens, like
the tetracyclines, can be used in the present compo-
sition and methods. Examples of useful antibiotics
20   include, but are not limited to, chlorhexidine,
chlorhexidine dihydrochloride, chlorhexidine
diacetate salt hydrate, chlorhexidine digluconate,
alexidine, alexidine dihydrochloride, tetracycline,
tetracycline hydrochloride, doxycycline, doxycycline
25   hydrochloride, and mixtures thereof.

The second active agent in a present com-
position and method is berberine. In humans,
berberine markedly improves cardiac performance in
patients with heart conditions when administered at
30   0.02 mg/kg/min (59 nmol/kg/min) for 30 minutes,
followed by 0.2 mg/kg/min (0.59 mol/kg/min) for an

additional 30 minutes. However, berberine admin-
istration to heart failure patients can result in
ventricular tachycardia in some subjects. Berberine
also has an anesthetic effect when injected s.c.,

5   and berberine produced parapsympatholytic and
anesthetic effects when applied to the eyes.

Berberine sulfate is absorbed through
human skin. Following oral administration, berber-
ine is absorbed slowly, requiring four hours to

10   reach peak concentrations in plasma and another four
hours to clear. In rats orally administered tri-
tiated berberine chloride, blood levels of the com-
pound leveled after 4 to 24 hours; peak levels in
liver and muscles occurred at 12 hours, while

15   urinary excretion peaked at 12 to 24 hours. After
48 hours, the majority of the administered dose had
been excreted in feces. Following intravenous
administration to rats, the highest concentrations
of berberine were found in the kidneys, with lower

20   concentrations in the liver, lung, and brain. In
rabbits, small amounts of berberine were found in
the heart, liver, and kidneys 24 hours after admin-
istration by gavage.

In mice, the oral LD_{50} dose of berberine is

25   329 mg/kg (0.98 mmol/kg), the s.c. LD_{50} dose is 18
mg/kg (0.054 mmol/kg), and the i.p. LD dose is
greater than 500 mg/kg (>1.49 mmol/kg (>2.31 mmol/-
kg) orally. In rabbits, the s.c. LD_{50} dose is 100
mg/kg (0.30 mmol/kg).

30   When tested as protection from amphetamine
toxicity, berberine chloride (5 mg/kg; 0.013 mmol/-

kg), and berberine sulfate (15 mg/kg; 0.035 mmol/-
kg), injected i.p. had no significant effect in
mice. Berberine sulfate reduced rectal temperature
in albino rats injected i.p. with 50 mg/kg (0.12

5 mmol/kg), and a single intraintestinal injection (10
mg/kg; 0.023 mmol/kg) into the duodenum of rats had
no effect on the volume or acidity intravenously at
6 mg/kg (0.014 mmol/kg). Berberine sulfate signifi-
cantly increased the number of apomorphine-induced

10 vomits in dogs, and reduced the blood pressure in
rats, dogs, and cats following intravenous adminis-
tration of 0.1-6.0 mg/kg (0.00023-0.014 mmol/kg).

A recent publication disclosed that
berberine administered in combination with pyrimeth-

15 amine gave good results in the treatment of patients
suffering from chloroquine-resistant malaria. It
also has been reported that berberine, isolated from
the Chinese medicinal plant, *Coptidis rhizoma*, has
antimicrobial activity against seven periodontal

20 pathogens, and that berberine, therefore, possibly
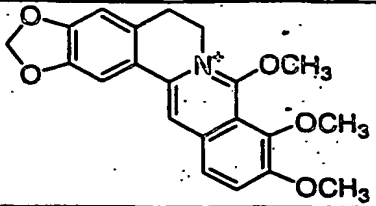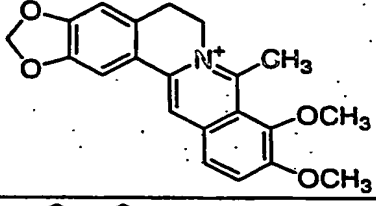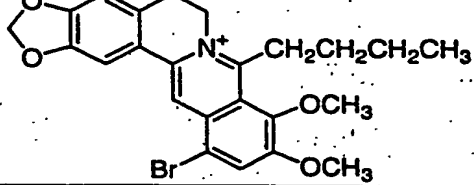can be used in a clinical treatment of periodontal
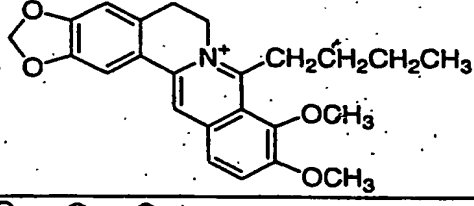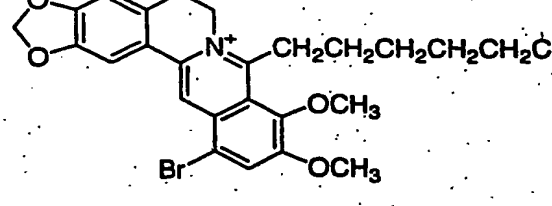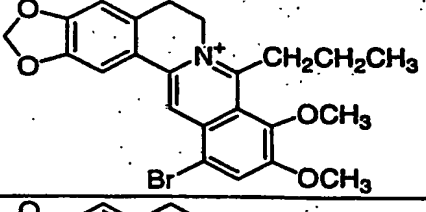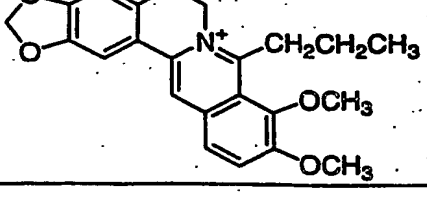diseases.

The present invention is directed to the
use of berberine, a natural compound found in

25 goldenseal, in combination with an antibiotic, e.g.,
CHX or a tetracycline, in the control of oral and
other human pathogens, and diseases and conditions
directly or indirectly caused by such pathogens.
The invention is not limited to the use of a par-

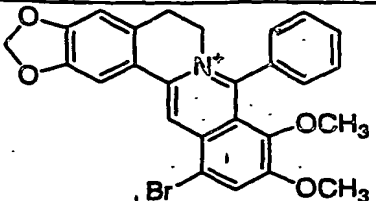30 ticular berberine, but extends to free berberine,
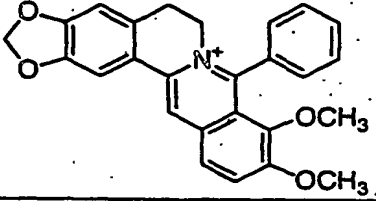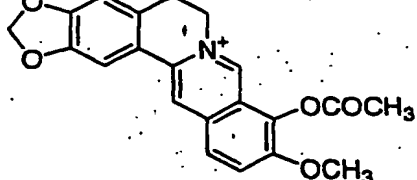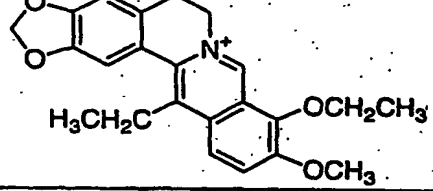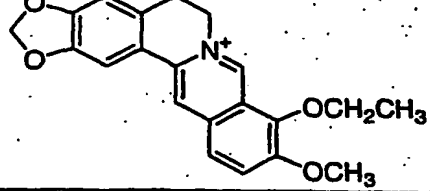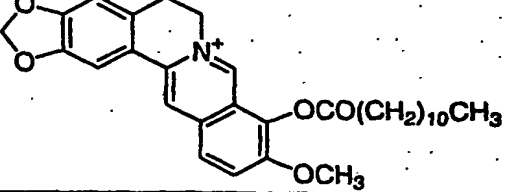
all pharmaceutically acceptable salts of berberine, derivatives of berberine, and prodrugs of berberine.
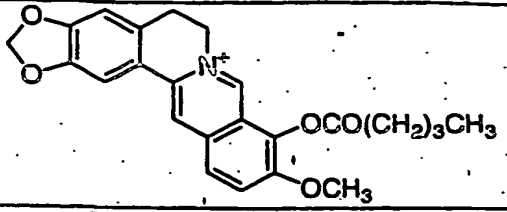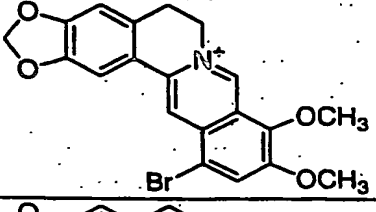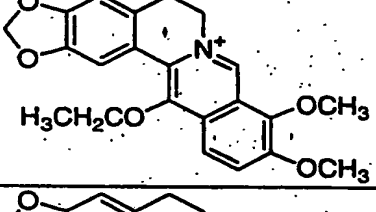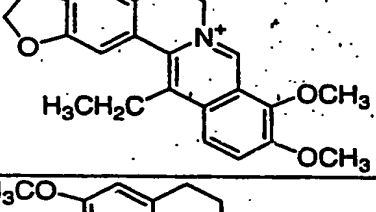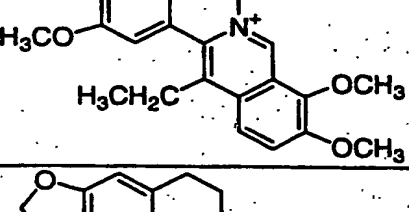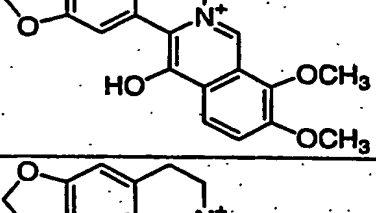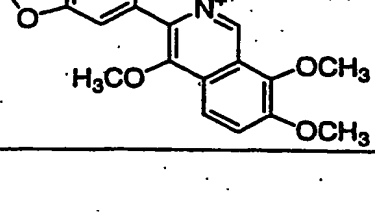
Examples of berberines and berberine derivatives useful in the present invention include,
5   but are not limited to, free berberine, berberine chloride, berberine bisulfate, berberine hemisulfate, a berberine prodrug, a berberine derivative listed in the following Table 1, and mixtures thereof.
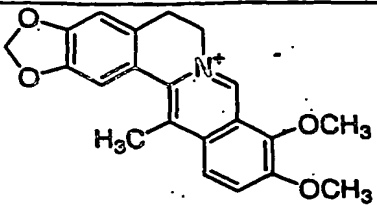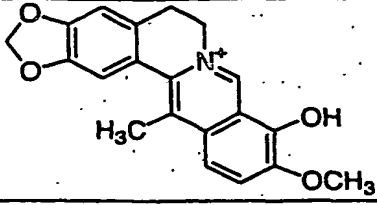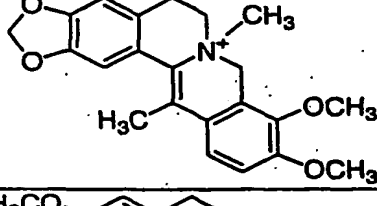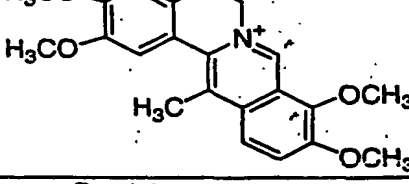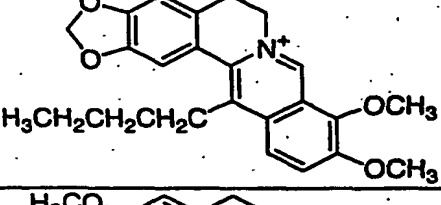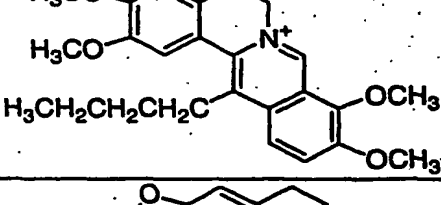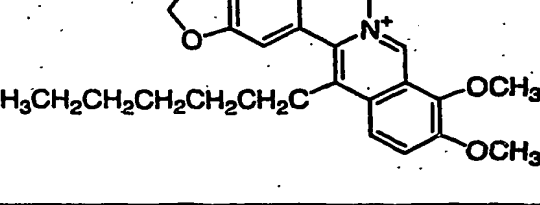
Table 1.

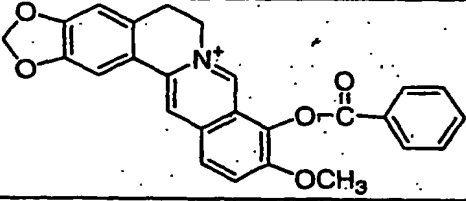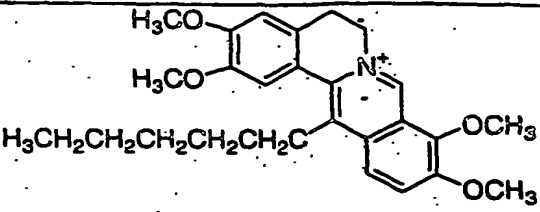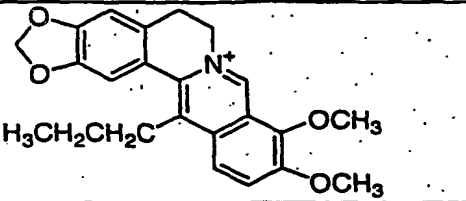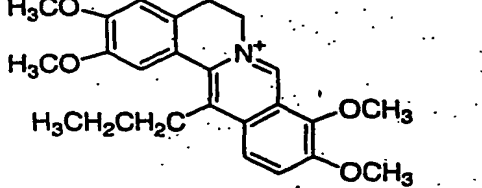| Name	Molecular Formula	Molecular Weight	Structure
berberine chloride	$C_{20}H_{18}ClNO_4$	371.81	
berberrubine	$C_{19}H_{16}NO_4$	322.33	
coptisine	$C_{19}H_{14}NO_4$	320.32	
palmatine	$C_{21}H_{22}NO_4$	352.40	
8-ethyl-12-bromoberberine	$C_{22}H_{21}BrNO_4$	443.31	
8-ethylberberine	$C_{22}H_{22}NO_4$	364.41	

8-methoxyberberine	$C_{21}H_{20}NO_5$	366.39	
8-methylberberine	$C_{21}H_{20}NO_4$	350.39	
8-n-butyl-12-bromoberberine	$C_{24}H_{25}BrNO_4$	471.36	
8-n-butylberberine	$C_{24}H_{26}NO_4$	392.47	
8-n-hexyl-12-bromoberberine	$C_{26}H_{29}BrNO_4$	499.42	
8-n-propyl-12-bromoberberine	$C_{23}H_{23}BrNO_4$	457.34	
8-n-propylberberine	$C_{23}H_{24}NO_4$	378.44	

8-phenyl-12-bromoberberine	$C_{26}H_{21}BrNO_4$	491.35	
8-phenylberberine	$C_{26}H_{22}NO_4$	412.46	
9-O-acetylberberrubine	$C_{21}H_{18}NO_5$	364.37	
9-O-benzoylberberrubine	$C_{26}H_{20}NO_5$	426.44	
9-O-ethyl-13-ethylberberrubine	$C_{23}H_{24}NO_4$	378.44	
9-O-ethylberberrubine	$C_{21}H_{20}NO_4$	350.39	
9-O-lauroylberberrubine	$C_{31}H_{38}NO_5$	504.64	

9-O-valerylberberrubine	$C_{24}H_{24}NO_5$	406.45	
12-bromoberberine	$C_{20}H_{17}BrNO_4$	415.26	
13-ethoxyberberine	$C_{22}H_{22}NO_5$	380.41	
13-ethylberberine	$C_{22}H_{22}NO_4$	364.41	
13-ethylpalmatine	$C_{23}H_{26}NO_4$	380.46	
13-hydroxyberberine	$C_{20}H_{18}NO_5$	352.36	
13-methoxyberberine	$C_{21}H_{20}NO_5$	366.39	

13-methylberberine	$C_{21}H_{20}NO_4$	350.39	
13-methylberberrubine	$C_{20}H_{18}NO_4$	336.36	
13-methyldihydroberberine N-methyl salt	$C_{22}H_{24}NO_4$	366.43	
13-methylpalmatine	$C_{22}H_{24}NO_4$	366.43	
13-n-butylberberine	$C_{24}H_{26}NO_4$	392.47	
13-n-butylpalmatine	$C_{25}H_{30}NO_4$	408.51	
13-n-hexylberberine	$C_{26}H_{30}NO_4$	420.52	

13-*n*-hexylpalmatine	$C_{27}H_{34}NO_4$	436.56	
13-*n*-propylberberine	$C_{23}H_{24}NO_4$	378.44	
13-*n*-propylpalmatine	$C_{24}H_{28}NO_4$	394.48	

Separately, berberine, chlorohexadine, and
tetracycline have been used as inhibitors of bacter-
ial growth. However, the present invention is the
first disclosure of a combined treatment using

5   berberine and an antibiotic, like chlorohexadine or
tetracycline, to treat gingivitis and other diseases
and conditions of the mouth, teeth, and gums. The
present invention also can be used to treat diseases
and conditions directly or indirectly caused by

10   nonoral human pathogens. When berberine is adminis-
tered in combination with an antibiotic, it has been
found that the dose of both the antibiotic and
berberine can be reduced by a factor of up to four
to achieve the same bacterial growth inhibition ob-

15   served using either an antibiotic or berberine
alone.

The present invention demonstrates that
berberine chloride, when administered with CHX or an
antibiotic, exerts synergistic growth inhibitory

20   activities. In recent years, oral hygiene products
and toothpastes containing goldenseal have become
widely available in the U.S. Antibiotics are used
in the treatment of periodontal disease. The pres-
ent invention allows the use of reduced concentra-

25   tions of CHX and a tetracycline in oral hygiene/-
therapeutic products to achieve a desired antimicro-
bial effect, thus minimizing the occurrence of ad-
verse effects attributed to antibiotics and berber-
ine and addressing the problem of pathogen resis-

30   tance to antibiotics.

The following tests, and tests described in Appendix A, were conducted to illustrate the synergistic potentiating effects achieved by coadministration of berberine and an antibiotic to a

5 mammal, including humans, to treat oral and other human pathogens.

When berberine hydrochloride was administered in combination with chlorhexidine gluconate (CHX) or a tetracycline antibiotic (e.g., tetra-

10 cycline hydrochloride or doxycycline hydrochloride), a synergistic microbial growth inhibition effect was observed. This active ingredient combination resulted in a lower minimum inhibitory concentration (MIC) compared to the MIC of either agent when

15 measured alone. Five different oral pathogens were evaluated for their susceptibility to combination of these active agents (*Streptococcus mutans, Streptococcus gordonii, Porphyromonas gingivalis, Fusobacterium nucleatum*, and *Anticobacillus actino-*

20 *mycetecomitans*). *A. actinomycetecomitans* was resistant to the test combinations when grown in aerobic conditions, but demonstrated susceptibility under anaerobic conditions.

Berberine chloride, purchased from Sigma

25 (St. Louis, MO), was tested to assess whether a different antimicrobial activity existed between a compound isolated from goldenseal and commercially available berberine chloride. The antimicrobial investigations showed no difference in antimicrobial

30 activity between the two sources of barbering chloride.

A combination treatment using berberine
hydrochloride and a conventional antimicrobial agent
allows for decreased concentrations of both agents
in the treatment of mouth, teeth, and gum conditions
5   and diseases, and reduces the occurrence of adverse
side effects. This is an important finding because
of the increase in resistance to conventional
antibacterial treatments. The present invention,
therefore, provides a new composition and method for
10 enhanced treatment of mouth, teeth, and gum diseases
and conditions, and for the treatment of other di-
seases and conditions caused, directly or indirect-
ly, by pathogens controlled by berberine and/or an
antibiotic.

15   In addition, two nonoral, human pathogens
(*Escherichia coli* and *Staphylococcus aureus*) also
were evaluated using the combination antibiotic-
berberine treatment. The test results showed that
when berberine hydrochloride was used in combination
20 with CHX or a tetracycline antibiotic (e.g., tetra-
cycline hydrochloride and doxycycline hydrochlor-
ide), a synergistic growth inhibition effect was
achieved. These findings show that a composition or
method of the present invention can have therapeutic
25 applications in addition to the treatment of oral
conditions and diseases.

          Berberrubine, structurally similar to
berberine, also was evaluated for antimicrobial
activity against *S. mutans*, *A. actinomycetecomitans*,
30 and *P. gingivalis*. The test results indicated that
berberrubine was not antimicrobial against *A.*

actinomycetecomitans at concentrations less than 300 µg ml^{-1}. The test results also suggest that berberrubine may be antimicrobial against *S. mutans* at concentrations greater than 300 µg ml^{-1} because,

5   at lower concentrations, growth was hindered, but not inhibited. Berberrubine demonstrated anti-microbial activity against *P. gingivalis*, at concentrations comparable to berberine (i.e., MIC 17 µg ml^{-1}).

10   The following table summarizes the approx-imate amount of an antibiotic, when combined with berberine hydrochloride, required to achieve a pre-determined inhibitory activity relative to using the antibiotic alone.

15

Species	CHX	Tetracycline (HCL)	Doxycycline (HCL)
S. mutans[1]	<1/2	1/4	1/4
S. gordonii[1]	<1/2	<1/4	<1/4
P. gingivalis[1]	<1/2	1/4	1/4
F. nucleatum[1]	<1/2	1/4	1/4
E. coli[2]	1/2	1/2	1/2
S. aureus[2]	1/2	1/4	1/5

[1] oral pathogen
[2] nonoral pathogen

20   The test results clearly demonstrate that the antimicrobial effects of an antibiotic and berberine on oral and other human pathogens are synergistically potentiated when the agents are co-administered, sequentially or simultaneously. This

25   is an important clinical finding because the amount of antibiotic and berberine required to achieve a desired antimicrobial effect can be substantially

24

reduced, which in turn substantially reduces adverse
effects attributed to antibiotic and berberine use.

    Berberine combined with an antibiotic,
therefore, can be used to potentiate the antimicro-
5   bial action of an antibiotic in the treatment of
mouth, teeth, and gum diseases, and other diseases
and conditions directly or indirectly caused by
human pathogens. The test results show that, when
combined with berberine administration, antibiotics
10   produce significant antimicrobial control using a
much lower dose of antibiotic and berberine, and,
therefore, bacterial resistance to the antibiotic
and berberine is reduced.

    The tests and data show that a combination
15   of an antibiotic and berberine can be administered
to mammals in methods of controlling oral and other
human pathogens, and treating pathogen-mediated
diseases and conditions, especially of the mouth,
teeth, and gums. The antibiotic and berberine
20   (i.e., active ingredients) can be formulated in
suitable excipients for oral administration, or for
parenteral administration. Such excipients are well
known in the art. The active agents typically are
present in such a composition in an amount of about
25   0.1% to about 75% by weight, either alone or in com-
bination.

    Pharmaceutical compositions containing the
active agents, i.e., antibiotic and berberine, of
the present invention are suitable for administra-
30   tion to humans or other mammals. Typically, the
pharmaceutical compositions are sterile, and contain

no toxic, carcinogenic, or mutagenic compounds that cause an adverse reaction when administered. Administration of the pharmaceutical composition can be performed before, during, or after the onset of a

5   disease or condition, e.g., gingivitis, endocarditis, or plaque formation.

The method of the present invention can be accomplished using the active agents as described above individually as the neat compounds, or as a

10 physiologically acceptable salt, solvate, derivative, or prodrug thereof. The active agents can be administered neat, or as a pharmaceutical composition containing either or both entities.

The active agents can be administered by

15 any suitable route, for example, by oral, topical, buccal, sublingual, nasal, percutaneous, i.e., transdermal, or parenteral (including intravenous, intramuscular, subcutaneous, and intracoronary) administration. Parenteral administration can be

20 accomplished using a needle and syringe, or using a high pressure technique, like POWDERJECT™. Typically, the active agents are administered orally, buccally, sublingually, or topically.

The pharmaceutical compositions include

25 those wherein the active ingredients are administered in an effective amount to achieve their intended purpose. More specifically, a "therapeutically effective amount" means an amount effective to prevent development of, to eliminate, or to

30 alleviate a mouth, teeth, gum, or other disease or condition. Determination of a therapeutically

effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

A "therapeutically effective dose" refers
5   to that amount of the active agents that results in achieving the desired effect. Toxicity and therapeutic efficacy of such active agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., determining
10   the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD_{50}
15   and ED_{50}. A high therapeutic index is preferred. The data obtained from such data can be used in formulating a range of dosage for use in humans. The dosage of the active agents preferably lies within a range of circulating concentrations that
20   include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed, and the route of administration utilized.

The exact formulation, route of adminis-
25   tration, and dosage is determined by an individual or physician in view of the individual's condition. Dosage amount and interval can be adjusted individually to provide levels of the active agents that are sufficient to maintain therapeutic or prophyl-
30   actic effects.

The amount of pharmaceutical composition
administered is dependent on the subject being
treated, on the subject's weight, the severity of
the affliction, the manner of administration, and
5   the judgment of the prescribing physician.

Specifically, for administration to a
human in the curative or prophylactic treatment of
oral and other human pathogens, dosages of an anti-
biotic and berberine, individually, generally are
10. about 10 to about 200 mg daily for an average adult
patient (70 kg), typically divided into two to three
doses per day or administered as a time-release
formulation. Thus, for a typical adult patient,
individual dosage forms contain about 0.1 to about
15   200 mg antibiotic and about 0.1 to about 200 mg
berberine, in a suitable pharmaceutically acceptable
vehicle or carrier, for administration in single or
multiple doses, once or several times per day. Dos-
ages for intravenous, buccal, topical, or sublingual
20   administration typically are about 0.1 to about 10
mg/kg per single dose as required. In practice, the
physician determines the actual dosing regimen which
is most suitable for an individual patient, and the
dosage varies with the age, weight, and response of
25   the particular patient. The above dosages are
exemplary of the average case, but there can be
individual instances in which higher or lower dos-
ages are merited, and such are within the scope of
this invention.

30   The active agents of the present invention
can be administered alone, or in admixture with a

pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. Pharmaceutical compositions for use in accordance with the present invention thus

5   can be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active agents into preparations which can be used pharmaceutically.

10   These pharmaceutical compositions can be manufactured in a conventional manner, e.g., by conventional mixing, dissolving, granulating, dragee-making, emulsifying, encapsulating, entrapping, or lyophilizing processes. Proper formulation

15 is dependent upon the route of administration chosen. When therapeutically effective amounts of the active agents are administered orally or topically, the composition typically is in the form of a tablet, capsule, powder, solution, or elixir.

20 When administered in tablet form, the composition can additionally contain a solid carrier, such as gelatin or an adjuvant. The tablet, capsule, and powder contain about 5% to about 95% of an active agent of the present invention, and preferably from

25 about 25% to about 90% compound of the present invention. When administered in liquid form, a liquid carrier, such as water, petroleum, or oils of animal or plant origin, can be added. The liquid form of the composition can further contain physi-

30 ological saline solution, dextrose or other saccharide solutions, or glycols. When administered in

liquid form, the composition contains about 0.5% to
about 90% by weight of active agents, and preferably
about 1% to about 50% of an active agent.

    The active agents can be readily combined

5   with pharmaceutically acceptable carriers well known
in the art. Such carriers enable the active agents
to be formulated as tablets, pills, dragees, cap-
sules, liquids, gels, syrups, slurries, suspensions,
and the like, for oral ingestion by a patient to be

10 treated. Pharmaceutical preparations for oral use
can be obtained by adding the active agents with a
solid excipient, optionally grinding the resulting
mixture, and processing the mixture of granules,
after adding suitable auxiliaries, if desired, to

15 obtain tablets or dragee cores. Suitable excipients
include, for example, fillers and cellulose prepara-
tions. If desired, disintegrating agents can be
added.

    The active agents can be formulated for

20 parenteral administration by injection, e.g., by
bolus injection or continuous infusion. Formula-
tions for injection can be presented in unit dosage
form, e.g., in ampules or in multidose containers,
with an added preservative. The compositions can

25 take such forms as suspensions, solutions, or emul-
sions in oily or aqueous vehicles, and can contain
formulatory agents such as suspending, stabilizing,
and/or dispersing agents.

    Pharmaceutical compositions for parenteral

30 administration include aqueous solutions of the
active agent in water-soluble form. Additionally,

suspensions of the active agents can be prepared as
appropriate oily injection suspensions. Suitable
lipophilic solvents or vehicles include fatty oils
or synthetic fatty acid esters. Aqueous injection
5   suspensions can contain substances which increase
the viscosity of the suspension. Optionally, the
suspension also can contain suitable stabilizers or
agents that increase the solubility of the compounds
and allow for the preparation of highly concentrated
10   solutions. Alternatively, a present composition can
be in powder form for constitution with a suitable
vehicle, e.g., sterile pyrogen-free water, before
use.

In addition to the formulations described
15   previously, the active agents also can be formulated
as a depot preparation. Such long-acting formula-
tions can be administered by implantation (for exam-
ple, subcutaneously or intramuscularly) or by intra-
muscular injection. Thus, for example, the active
20   agents can be formulated with suitable polymeric or
hydrophobic materials (for example, as an emulsion
in an acceptable oil) or ion exchange resins, or as
sparingly soluble derivatives, for example, as a
sparingly soluble salt.

25   In particular, the active agents can be
administered orally, buccally, or sublingually in
the form of tablets containing excipients, such as
starch or lactose, or in capsules or ovules, either
alone or in admixture with excipients, or in the
30   form of elixirs of suspensions containing flavoring
or coloring agents. Such liquid preparations can be

prepared with pharmaceutically acceptable additives,
such as suspending agents. A compound also can be
injected parenterally, for example, intravenously,
intramuscularly, subcutaneously, or intracoronarily.

5   For parenteral administration, the compound is best
used in the form of a sterile aqueous solution which
can contain other substances, for example, salts, or
monosaccharides, such as mannitol or glucose, to
make the solution isotonic with blood.

10   For veterinary use, the active ingredients
are administered as a suitably acceptable formula-
tion in accordance with normal veterinary practice.
The veterinarian can readily determine the dosing
regimen and route of administration that is most

15 appropriate for a particular animal.

           As stated above, an antibiotic is widely
used in the treatment of several disease conditions
of the mouth and gums, including gingivitis. A
major problem in the use of an antibiotic in an oral

20 application is their adverse side effects. The
problem of bacterial resistance to an antibiotic
also exists. It has been discovered that using
berberine together with an antibiotic synergisti-
cally potentiates the antimicrobial action of the

25 antibiotic, thereby allowing the administration of a
reduced amount of antibiotic and berberine to
achieve a desired result while addressing bacterial
resistance associated with antibiotic and berberine
use.

30   To date, no published literature exists
disclosing the synergistic effect observed when

berberine is used in combination with a conventional antimicrobial agent, such as CHX, or antibiotics, such as a tetracycline, against oral and other human pathogens. The present invention, therefore, is

5 directed to new drug therapies, such as sustained release systems and new oral hygiene compositions.

In addition, goldenseal is a relatively rare plant and only a small portion of commercially available goldenseal is derived from cultivated

10 crops. The present invention is important with respect to identifying the active components of goldenseal that are formulated into efficacious products, such as oral hygiene products. Berberine derivatives or prodrugs also can be used in accord-

15 ance with the present invention to overcome availability problems associated with naturally occurring berberine and to reduce the costs associated with extracting berberine from plant sources. The synergistic effect of the combined application of berber-

20 ine and antibiotic also applies to other human pathogens and, therefore, the combination can be used in the treatment of nonoral human conditions and diseases.

In particular, a present composition and

25 method can be used in the following, nonlimiting practical applications:

(1) over-the-counter oral hygiene products (e.g., mouth rinses and dentrifrices) for the control of plaque and gingivitis using a combination

30 of a berberine and an antibiotic;

(2)   the combination of berberine chloride
and an antibiotic, like CHX, can be used in other
oral products, including dental adhesives for ortho-
dontic brackets to reduce plaque accumulation,
5   dental impression material, chewing gum for caries
and gingivitis control, press mints, and varnishes;

(3)   a therapeutic treatment or an adjunct
for treatment of periodontitis.

Modifications and variations of the inven-
10   tion as hereinbefore set forth can be made without
departing from the spirit and scope thereof, and,
therefore, only such limitations should be imposed
as are indicated by the appended claims.

APPENDIX A

INTERACTIONS BETWEEN ALKALOIDS EXTRACTED FROM *HYDRASTIS CANADENSIS* AND CONVENTIONAL ANTIMICROBIAL AGENTS

Hydrastis canadensis L. (Ranunculaceae), a North American plant known commercially as "goldenseal," has emerged as one of the top five herbal supplements in the worldwide market (Govindan and Govindan, 2000). Goldenseal is used in various dietary supplements for the purpose of enhancing the immune response of the body. Goldenseal-containing oral hygiene products are sold over the counter (OTC). However, limited information exists with respect to the efficacy of goldenseal components that contribute to purported oral health benefits (McCann et al., 2000).

Berberine is a plant alkaloid having a long history of medicinal use in both Ayurvedic and Chinese medicine. Berberine is present in *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). Berberine can be found in the roots, rhizomes, and stem bark of these plants. Berberine extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms, including bacteria, viruses, fungi, protozoans, helminths, and chlamydia (Park et al., 2001; Cowan, 1999; Grippa et al., 1999; Park et al., 1999). The amount of berberine in OTC products is about 50 to 200 mg. Currently, the predominant clinical uses for berberine include treatment of

bacterial diarrhea, intestinal parasite infections,
and ocular trachoma infections (Harry et al., 1988).

　　　　Chlorhexidine (CHX) is a bisbiguanide bio-
cide, and compositions containing CHX have been used
as topical disinfectants since the middle 1970s.
CHX targets the cytoplasmic membrane of bacteria re-
sulting in the loss of structural organization and
integrity, and ultimately the coagulation and pre-
cipitation of cytoplasmic constituents. In peri-
odontal therapy, CHX has been used in postoperative
rinsings, subgingival irrigation, and as an adjunct
to mechanical debriment. CHX has a low level of
toxicity, both locally and systemically, and shows
no permanent retention in the body (International
Health Care Foundation, 2001). CHX usually is de-
livered in rinsing solutions (typically containing
1-2% CHX), and slow-release devices such as var-
nishes and slab-like sustained delivery devices
(Southard and Godowski, 1998). CHX also can be
incorporated into chewing gum, toothpastes, and
toothbrushes, or as a disinfectant for dentures in
patients with candidal infections (International
Health Care Foundation, 2001).

　　　　Tetracyclines are broad-spectrum anti-
biotics that can be delivered by many routes of
administration, including oral administration and
sustained delivery systems. Tetracyclines inhibit
protein synthesis by combining with the small (30S)
subunit of the ribosome and inhibiting the binding
of aminoscyl-tRNA molecules to the ribosomal A site
(Prescott et al., 1999). Tetracyclines generally
are considered bacteriostatic. The number of re-

ports directed to bacterial resistance to tetra-
cyclines, both *in vitro* and *in vivo*, is increasing,
however, tetracyclines still are very useful and are
routinely used in periodontal treatment. However,
if resistant bacteria constitute potential patho-
gens, the periodontal disease may not be treated,
but rather aggravated further (Slots and Rams,
1990).

Tetracyclines concentrate in periodontal
pockets at inhibitory levels 2-4 times higher than
in the blood. Tetracyclines also strongly bind to
root surfaces and can be released in active form
over extended time periods. Sublethal concentra-
tions of tetracyclines reduce adherence and coaggre-
gation properties of a number of disease-associated
bacteria, and also inhibit the collagenolytic en-
zymes of mammalian cells enabling periodontal lig-
ament regeneration (Slots and Rams, 1990).

The present invention shows: a) the anti-
microbial activity of goldenseal alkaloids against
representative oral pathogens and human pathogens,
and b) the synergistic efficacy of berberine admin-
istration combined with administration of an
antibiotic, like CHX, tetracycline (Tet), colistin
sulfate (Col), or doxycycline (Dox), against oral
and other human pathogens.

MATERIALS AND METHODS

Extraction of alkaloids

The alkaloids extracted from goldenseal
were berberine, hydrastine, canadaline, canadine and

isocorypalmine. A crude extract also was obtained.
A berberine derivative, i.e., berberrubine, also was
tested, but was not isolated from goldenseal.
Berberine and hydrastine were formulated as a chlor-
ide salt and a hydrochloride salt, respectively,
which eliminated solubility problems associated with
the original compounds.

Growth conditions

Five representative oral pathogens were
used as the test organisms, i.e., *Streptococcus
mutans*, *Streptococcus gordonii*, *Porphymonas gingi-
valis*, *Fusobacterium nucleatum*, and *Actinobacillus
actinomycetemcomitans*. Cultures were obtained from
the University of Illinois-Chicago culture collec-
tion at the College of Dentistry. *S. mutans*, *S.
gordonii*, and *A. actinomycetemcomitans* were grown in
Brain Heart Infusion broth (Difco, Becton Dickinson
and Co., Sparks, MD, USA). *P. gingivalis* was grown
using defined media containing (l^{-1}):30 g Tryptic Soy
Broth (Difco, USA); 5 g Yeast Extract (Difco, USA);
0.5 g 1-cysteine hydrochloride (Sigma, USA); 0.3 µg
menadione (Sigma, USA) and 5 µg hemin (Sigma, USA).
F. nucleatum was grown in Schaedler Broth (Difco,
USA). Three human pathogens also were tested,
Escherichia coli, *Staphylococcus aureus*, and *Pseu-
domonas aeruginosa*. Cultures were obtained from the
University of Illinois-Chicago culture collection at
the College of Medicine. *E. coli*, *St. aureus*, and
P. aeruginosa were grown in Luria-Bertani (LB) broth
(Difco, USA).

Antibacterial determination

The antimicrobial activity of the alka-
loids (as minimum inhibitory concentrations, or MIC)
were evaluated using a microdilution assay in 96-
well microtiter plates (Fisher Scientific, USA).
The tested compounds were berberine (extracted from
goldenseal, B-G), berberine chloride (commercial,
Sigma, USA, B-S), berberine chloride goldenseal (B-
CG), canadaline, hydrastine, hydrastine chloride,
canadine, isocorypalmine, berberrubine (against *S.
mutans*, *A. actinomycetemcomitans*, and *P. gingivalis*
only at concentrations of 0-300 $\mu g \cdot ml^{-1}$), and the
crude extract (at concentrations of 0-500 $\mu g\ ml^{-1}$).
CHX was used as a positive control at concentrations
of 0.02 to 10 $\mu g\ ml^{-1}$. Only berberine (B-G and B-S)
was tested against *E. coli*, *St. aureus*, and *P. aeru-
ginosa*.

Overnight cultures of each species were
centrifuged (2400 x g, 10 minutes), washed twice
with 0.05 M phosphate buffered saline (PBS, pH 6.8),
and re-suspended. The cell suspension was adjusted
using a Cecil Spectrophotometer Series 601 (Milton
Roy, Rochester, NY) giving a final concentration in
each well of 5 x 10^5 colony forming units (CFU) ml^{-1}
for the aerobic species and 5 x 10^6 CFU ml^{-1} for the
anaerobic species, in accordance with NCCLS recom-
mendations. All plates were incubated at 37°C under
appropriate atmospheric conditions. *S. mutans* and
S. gordonii were incubated aerobically (also *E.
coli*, *S. aureus*, and *P. aeruginosa*). *F. nucleatum*
and *P. gingivalus* were incubated anaerobically
(anaerobic chamber, Ionia Scientific Inc., Marietta,

OH) in 10% H_2, 5% CO_2, and 85% N_2. *A. actino-
mycetemcomitans* was incubated both aerobically and
anaerobically. Growth was monitored spectrophoto-
metrically (A_{660nm}) at 0, 24, and 48 h using a Power
Wave 200 Microplate Scanning Spectrophotometer
(BioTek Instruments, USA). The readings were cap-
tured and analyzed using the associated KC4 soft-
ware, Version 2, Revision 12 (BioTek Instruments,
USA). Because berberine and other compounds, for
example, berberrubine, are colored, the MIC of the
test compounds was defined as the minimum concen-
tration of compound limiting turbidity equal to the
compound color blank. The compound controls in-
cluded inoculated growth medium without test com-
pounds.

**Determination of Minimum Bactericide
Concentrations (MBC)**

The MBC of the compounds against each test
species was performed and determined as described in
the previous section for MIC determination. After
48 h, 100 µl of culture was removed from wells con-
taining the compound concentrations at or higher
than the MIC. Serial dilutions (10^{-1} to 10^{-5}) were
performed for each sample, and 100 µl of the appro-
priate dilutions was plated out in triplicate onto
appropriate agar. After 48 h, and incubation at
37°C under appropriate atmospheric conditions, the
number of CFU ml^{-1} were enumerated. The MBC was
defined as the lowest concentration of compound that
killed 99.9 % of the test bacterial population.

Combination determination

The alkaloids were tested in pair-wise
combinations (fractional inhibitory concentrations,
FIC) against all the bacteria to determine whether
interactions were antagonistic (FIC>1), additive
(0.5<FIC<1), or synergistic (FIC<0.5). The FIC was
calculated by the following: (MIC $A_{combination}$/MIC
A_{alone}) + (MIC $B_{combination}$/MIC B_{alone}). Where A repre-
sents compound 1 and B represents compound 2. The
antibacterial efficacy of the alkaloids also were
tested in combination with tetracycline HCl (Tet·
HCl) (0.2 to 25 µg ml^{-1}) (Sigma Chemicals, St. Louis,
MO), doxycycline HCl (Dox HCl) (0.2 to 25 µg ml^{-1})
(Sigma, USA), and CHX (0.02 to 10 µg ml^{-1}) (Sigma,
USA). In the case of *A. actinomycetemcomitans* and
P. aeruginosa, CHX was used at 0.78 to 50 µg ml^{-1}.
Berberine (B-CG and B-S) was used at concentrations
0.9 to 250 µg ml^{-1}. The antibacterial efficacy of
berberine (1.95 to 500 µg ml^{-1}) was tested in combi-
nation against *P. aeruginosa* with CHX (0.78 to 50 µg
ml^{-1}) (Sigma Chemicals) and the polymyxin antibiotic
Col (0.04 to 25 µg ml^{-1}) (Sigma Chemicals). The
plates were incubated for 24 hours.

Viability assessment

Two representative organisms, *S. mutans*
and *F. nucleatum*, were used in this study. Viabil-
ity of *S. mutans* to berberine (B-CG) 500 and 1000 µg
ml^{-1}); CHX (20 µg ml^{-1}); B (500 µg ml^{-1}) with CHX (10
µg ml^{-1}); and B (500 µg ml^{-1}) with CHX (5 µg ml^{-1}) was
tested. Viability of *F. nucleatum* to B (500 and

1000 µg ml^{-1}); Dox HCL (5 µg ml^{-1}); B (500 µg ml^{-1})
with Dox (2.5 µg ml^{-1}); and B (250 µg ml^{-1}) with Dox
(2.5 µg ml^{-1}) also was tested. The bacteria were
prepared as previously described and the optical
density was adjusted to give a concentration 1 x 10^9
CFU ml^{-1}. Aliquots of 4 ml of agent, prepared in
sterile water, were placed into sterile test tubes
and 1 ml of adjusted cells was added. The control
consisted of 4 ml of PBS and 1 ml of cells. Samples
were removed at 0, 15, 30, and 60 minutes, serial
dilutions were performed, and the appropriate dilu-
tions plated out. The plates were incubated at 37°C
under appropriate atmospheric conditions for up to
72 hours. Viable colony counts were determined and
compared to nontreated control Percentage kill was
calculated using the formula:

$$(CFU_{control} - CFR_{treat})/CFU_{control} \times 100.$$

Log reduction was also calculated on Log$_{10}$ trans-
formed data.

Effect of berberine on cell surface hydrophobicity

Relative cell surface hydrophobicity of
the test bacteria was evaluated using a modified
method based on Rosenberg et al. (1980). In brief,
the cells were collected by centrifugation (2400 x
g, 10 minutes), washed twice with phosphate buffered
saline (PBS) (0.05 M, pH 6.8), adjusted to give a
concentration of 1 x 10^8 CFU ml^{-1}, and mixed with
equal volumes of the test agents (Table 1). After
standing for 10 minutes at room temperature, 500 µl
of hexanes (Sigma Chemicals) was added and the re-
action mixture vigorously agitated for 1 minute.

After separating the organic phase was separated from the aqueous phase, the optical density (O.D. A_{550}nm) of the aqueous phase was determined. The relative cell surface hydrophobicity was expressed as a percentage of the absorbance of the organic phase

(O.D.$_{\text{Initial Aqueous}}$-O.D.$_{\text{Extracted Organic}}$)/O.D.$_{\text{Initial Aqueous}}$ X 100).

Table 1 Concentration of agents used for the hydrophobicity assay			
Agent		Concentration (μg ml^{-1})	
Control		0	
Berberine (B)		500	
CHX		5	
Tet		5	
Dox		5	
B	CHX	125	1.25
B	Tet	125	1.25
B	Dox	125	1.25

Accumulation and efflux of berberine

An assay based on the ability of *S. mutans*, *S. gordonii*, and *A. actinomycetemcomitans* to grow in the presence of methylene blue (Morita et al., 1998), a substrate for the *acrAB* efflux system was used to screen for efflux activity. These organisms were used because of their higher susceptibility or resistance to berberine. The bacteria were grown on a series of agar plates containing BHI, and/or berberine (62.5, 125, and 250 μg·ml^{-1}) and/or methylene blue (50 μg ml^{-1}), and were incubated for 48 hours at 37°C.

Accumulation of berberine in *A. actinomycetemcomitans* was evaluated using a fluorimetric

assay (based in Giraud et al., 2000). Overnight
cells were harvested as previously described, washed
twice with 50 mM sodium phosphate buffer (pH 7),
resuspended in the same buffer at a concentration of
1×10^9 CFU ml^{-1}. The cells were equilibrated for 10
minutes at 37°C. Berberine was added at a final
concentration of 500 µg ml^{-1}. After addition of
berberine, 0.5 ml samples were removed at different
time intervals. Five minutes after the addition of
berberine, the efflux pump inhibitor carbonyl cya-
nide m-chlorophenylhydrazone (CCCP) was added to the
reaction mixture (final concentration 100 µM). The
samples were immediately diluted with 1 ml ice-cold
sodium phosphate buffer and then were centrifuged
for 5 minutes at 5600 x g. The pellet was washed
once with 1 ml ice-cold buffer and resuspended in 1
ml of 0.1 M glycine hydrochloride (pH 3) for at
least 15 hours at room temperature. The fluores-
cence of the supernatant was measured (excitation
355 nm and emission 517 nm) with a spectrofluori-
meter (Model 810/814, Photon Technology Inter-
national, Monmouth Junction, NJ).

Statistical analysis

All experiments were repeated at least
four times. Statistical analyses were carried out
using Microsoft Excel 1997. Descriptive statistics
including the mean, standard deviation, coefficient
((SD/mean) x 100) of variation were calculated. The
results were analyzed for significance using one-way
analysis of variance and the post-hoc Tukey's test
(ANOVA, $P < 0.05$; MINITAB® 2002, Version 13.32).

Linear regression analysis was performed using
Microsoft Excel 1997.

Results

Antibacterial determination

        Inhibitory effects against all the bac-
teria, except *A. actinomycetemcomitans*, were ob-
served for berberine (B-CG, B-G, and B-S) and the
whole extract (WE) (Table 2). There was no differ-
ence in antimicrobial activity between B-CG and the
commercially available berberine (B-S). A clear
difference in susceptibility to berberine was
observed among the test species. *P. gingivalis* and
F. nucleatum exhibited greatest susceptibility to
berberine and WE, i.e., eight to sixteen times lower
than that of the *Streptococcal* strains. Hydrastine,
the major component (2-6% w/w) of goldenseal, did
not inhibit growth of the test bacteria at concen-
trations ≤ 500 µg ml^{-1}. Canadaline, hydrastine,
canadine, and isocorypalmine did not exhibit anti-
microbial properties at concentrations ≤ 250 µg ml^{-1}.
A. actinomycetemcomitans was resistant to the crude
extract and all the test compounds at concentrations
≤ 500 µg ml^{-1}.

        Berberrubine, an analog of berberine, did
not inhibit the growth of *A. actinomycetemcomitans*
at concentrations ≤ 300 µg ml^{-1}. However, berber-
rubine may be antimicrobial against *S. mutans* at
concentrations higher than 300 µg ml^{-1} because at
lower concentrations growth was limited but not
inhibited. Berberrubine demonstrated antimicrobial

activity against *P. gingivalis* at concentrations
comparable to berberine (MIC 17 µg ml^{-1}).

Table 2. MIC (ug ml^{-1}) of Goldenseal Alkaloids Against Selected Oral Pathogens					
	Test Bacteria				
Sample	Sm	Sg	Aa	Fn	Pg
Berberine	125	250	>500	15.625	15.625
Hydrastine	>500	>500	>500	>500	>500
Canadine	>500	>500	>500	>500	>500
Canadaline	>250	>250	>250	>250	>250
Isocorypalamine	>125	>125	>125	>125	>125
Whole Extract (WE)	250	500	>500	62.5	62.5
CHX	1.25	5	50	2.5	2.5

Sm-*S. mutans*; Sg-*S. gordonii*; Aa-*A. actinomycetemcomitans*; Fn-
F. nucleatum, and Pg-*P. gingivalis*

This assay system allows concomitant eval-
uation of the minimum concentration that inhibits
biofilm growth. The MIC values obtained for each
species also represent the concentration that inhib-
ited biofilm formation. There was no difference in
antimicrobial activity between B-G and the other
berberine compounds (B-S and B-CG).

**Antimicrobial activity of berberine in
combination with CHX or tetracyclines**

Combinations of B-CG with CHX, Tet, Dox,
Col yielded lower a MIC than when either agent was
used alone (Table 3 and Table 4). Growth of *P.
aeruginosa* was not completely inhibited by exposure
to berberine, although growth was impaired at the
highest concentration (500 µg ml^{-1}). There was no
clear additive or synergistic effect observed by
combining berberine with either CHX or Col (Table
3).

Table 3 MIC (µg ml^{-1}) of the antimicrobial agents alone					
Species	Berberine	CHX	Tet	Dox	Col
S. mutans	125	1.25	1.56	0.78	ND
S. gordonii	250	5	0.31	0.31	ND
P. gingivalis	23.4	1.25	0.08	0.08	ND
A. actinomycetemcomitans*	>500	50	25	6.25	ND
F. nucleatum	15.6	2.5	0.16	0.04	ND
E. coli	250	1.25	3.06	1.53	ND
S. aureus	250	2.5	1.53	1.53	ND
P. aeruginosa	>500	6.25	ND	ND	3.13

Table 4 MIC (µg ml^{-1}) of the antimicrobial agents in combination					
Species	Berberine	CHX	Tet	Dox	Col
S. mutans	62.5	0.04	0.20	0.20	ND
S. gordonii	62.5	0.63	0.08	0.08	ND
P. gingivalis	7.81	0.08	0.01	0.01	ND
A. actinomycetemcomitans*	250	50	12.5**	6.25	ND
F. nucleatum	3.9	0.31	0.02	0.10	ND
E. coli	125	0.63	1.53	0.77	ND.
S. aureus	62.5	0.63	0.39	0.39	ND
P. aeruginosa	250**	3.13**	ND	ND	1.56

MIC values represent the mean of at least three separate
replicates

*Grown under anaerobic conditions

ND--not determined

** These values represent borderline additive/synergistic
interactions

        Because berberine (B-CG and B-S) was the
only compound that demonstrated antimicrobial
activity, no fractional inhibitory concentrations
were obtained for the remaining compounds.
        The results show that a synergistic growth
inhibition effect was observed (Table 4) when
berberine (B-CG and B-S) was used in combination
with CHX or tetracycline antibiotics (including

tetracycline hydrochloride and doxycycline hydro-
chloride). Typical results of FIC determination are
summarized in Table 5. These combinations resulted
in a lower minimum inhibitory concentration (MIC) of
either agent when measured alone (Table 6).

Table 5. Typical results from the FIC determination. This sample demonstrates the response of *S. mutans* to combinations of berberine (B-G) and CHX after 48 h incubation at 37°C											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.063	0.058	0.057	0.059	0.059	0.062	0.062	0.061	0.319	0.062	0.053	0.048
B	0.054	0.052	0.052	0.053	0.056	0.053	0.054	0.058	0.342	0.056	0.051	0.050
C	0.051	0.052	0.051	0.053	0.054	0.062	0.195	0.266	0.340	0.136	0.051	0.051
D	0.053	0.052	0.052	0.053	0.453	0.355	0.343	0.344	0.354	0.328	0.059	0.050
E	0.050	0.050	0.051	0.052	0.433	0.360	0.343	0.348	0.350	0.345	0.428	0.051
F	0.049	0.053	0.050	0.052	0.433	0.349	0.358	0.351	0.346	0.338	0.351	0.051
G	0.051	0.051	0.051	0.053	0.433	0.346	0.351	0.346	0.352	0.354	0.364	0.052
H	0.331	0.336	0.332	0.329	0.341	0.354	0.352	0.339	0.341	0.352	0.359	0.051

CHX was diluted from left to right at 10
to 0.08 µg ml^{-1} (rows 1 to 8), berberine was diluted
from top to bottom at 250 to 0.09 µg ml^{-1} (columns 1
to 8). Column 9 was the cell control; column 10 was
berberine alone (250 to 0.09 µg ml^{-1}); and column 11
was CHX alone (10 to 0.08 µg ml^{-1}). Row H was the
solvent control (8 % DMSO, H1 to H8). Column 12 was
the media blank. The dilution assay was designed
such that A1 contained the highest concentration of
both agents (berberine 250 µg ml^{-1} and CHX 10 µg
ml^{-1}). The final concentration of berberine in wells
A2 to A8 was 125 µg ml^{-1}. The FIC value was calcu-
lated from well C6. FIC was calculated by: (MIC
A$_{combination}$/MIC A$_{alone}$) + (MIC B$_{combination}$/MIC B$_{alone}$). For
C6, A represents berberine and B represents CHX:
Berberine (31.25/125) + CHX (0.3125/1.25) = FIC of
0.5, exhibiting a synergistic response.

Table 6. Approximate amount of CHX, tetracycline, or doxycycline required when combined with berberine (B-CG and B-S) relative to when the agent is used alone			
Species	CHX	Tetracycline-HCl	Doxycycline-HCl
S. mutans	< ½	¼	¼
S. gordonii	< ½	< ¼	< ¼
P. gingivalis	< ½	¼	¼
F. nucleatum	< ½	¼	¼
E. coli	½	½	½
S. aureus	½	¼	½

Viability assessment

Viability of both *S. mutans* and *F. nucleatum* was reduced by exposure to B-CG and was dependent on concentration and exposure time. This was most apparent for *F. nucleatum* (Table 7). Viability of *F. nucleatum* continued to decline during exposure to B-CG throughout the experimental period. In comparison, B-CG was most active during the first 15 minutes of exposure against *S. mutans*. The activity of B-CG was comparable to CHX against *S. mutans* and *F. nucleatum* (Table 7, $P>0.05$).

Table 7 Bactericidal activity of berberine against *S. mutans* and *F. nucleatum*			
Species	Viability after 60 min exposure (mean Log_{10} reduction of CFU)		
	B-CG (500)	B-CG (1000)	CHX (20)
S. mutans	0	1*	1.69*
F. nucleatum	2.77*	3.22*	3.09*

* Indicates a significant reduction compared to the control at a 95% confidence interval.

Combining B-CG and CHX yielded a reduction in viability that was comparable to viability reduction when the agents were used alone at higher

concentrations against *S. mutans* as shown in Table 8 and Table 9 ($P<0.05$).

Table 8 Bactericidal activity of combinations of B-CG and CHX against *S. mutans*					
	Viability after 60 min exposure (mean Log_{10} reduction of CFU)				
Species	B-CG (500)	B-CG (1000)	CHX (20)	B-CG (500) & CHX (10)	B-CG (500) & CHX (5)
S. mutans	0	1	1.69	1.18	0.22

Table 9 Comparison of mean Log_{10} reduction in viability of *S. mutans* after 60 min exposure evaluated using Tukey's post-hoc comparison test				
	B-CG (500)	B-CG (1000)	CHX (20)	B-CG (500) & CHX (10)
B-CG (1000)	NSD			
CHX (20)	SD	NSD		
B-CG (500) & CHX (10)	NSD	NSD	NSD	
B-CG (500) & CHX (5)	NSD	NSD	SD	NSD

SD indicates a significant difference in the reduction of viability between treatments at a 95% confidence interval.

NSD indicates no significant difference in the reduction of viability between treatments at a 95% confidence interval.

Combining B-CG and Dox yielded a reduction in viability that was comparable to viability reduction when the agents were used alone at higher concentrations against *F. nucleatum* as shown in Table 10 and Table 11 ($P<0.05$). The concentration required to achieve comparable reduction in viability when the agents were used in combination was reduced one-half for Dox (5 µg ml^{-1}) and four times for berberine (250 µg ml^{-1}) (Table 10 and Table 11).

Table 10 Bactericidal activity of combinations of B-CG with Dox against *F. nucleatum*					
	Viability after 60 min exposures (mean Log_{10} reduction of CFU)				
Species	B-CG (500)	B-CG (1000)	Dox (10)	B-CG (250) & Dox (5)	B-CG (250) & Dox (2.5)
F. nucleatum	2.77	3.22	1.56	3.37	2.09

Table 11 Comparison of mean Log_{10} reduction in viability of *F. nucleatum* after 60 min exposure evaluated using Tukey's post-hoc comparison test				
	B-CG (500)	B-CG (1000)	Dox (10)	B-CG (500) & Dox (5)
B-CG (1000)	NSD			
Dox (10)	NSD	NSD		
B-CG (250) & D9x (5)	NSD	NSD	SD	
B-CG (250) & Dox (2.5)	NSD	NSD	NSD	NSD

SD indicates a significant difference in the reduction of
viability between treatments at a 95% confidence interval.

NSD indicates no significant difference in the reduction of
viability between treatments at a 95% confidence interval.

Effect of berberine on cell surface hydrophobicity

The reduction of relative surface hydro-
phobicity is outlined in Table 12. B-CG was shown
to reduce hydrophobicity between 15% and 30%. CHX,
Tet, and Dox did not appear to affect the relative
hydrophobicity of the cells. Reduction in cell
surface hydrophobicity still was observed, however,
when the agents were tested in combination.

The assay demonstrated that *A. actino-
bacillus* was less hydrophobic compared to the other
bacteria. When *A. actinobacillus* was exposed to the
agents in combination and alone, an increase surface
hydrophobicity of about 25% was observed.

Table 12 Typical reduction scores (%) on relative cell surface hydrophobicity					
Treatment	S. mutans	S. gordonii	F. nucleatum	A. actinomycetemcomitans	P. gingivalis
B-500	14.8	26.1	29.9	-31.6	26.7
Tet-5	4.3	9.7	0.4	-21.55	0.7
Dox-5	8.1	7.7	-3.5	-4.1	1.7
CHX-5	5.1	-6.3	3.7	-2.6	-2.7
B-CHX (125 & 1.25)	7.2	19.8	26.1	-26.1	14.3
B-Tet (125 & 1.25)	9.4	18.6	28.2	-24.7	16.4
B-Dox (125 & 1.25)	9.4	23.4	28.9	-24.8	23.5

Accumulation and efflux of berberine

S. mutans and S. gordonii did not grow in
the presence of methylene blue (MB) (50 µg/ml) and
only grew in the presence of B-CG at 62.5 µg/ml. A.
actinomycetemcomitans grew in the presence of B-CG
at all concentrations at MB. When efflux of berber-
ine by A. actinomycetemcomitans was monitored spec-
trofluorometrically an initial accumulation was
observed followed by a decline (Fig. 1). Addition
of CCCP induced a very rapid and marked increase in
berberine accumulation (Fig. 1).

Discussion

To date there is no published reference
disclosing the enhanced effect, observed when berber-
ine is used in combination with a conventional anti-
microbial agents, such as CHX, or antibiotics, such
as tetracyclines, against oral and human pathogens.

With the increase in the number of reports
of bacterial resistance to conventional treatment,
attention now is turning to the management of infec-
tions with nonconventional antimicrobials (Kristian-
sen et al., 1997; Appleman et al., 2000).

Plants remain a major source of medicinal
compounds. It is estimated that 20,000 plant spe-
cies are used for medicinal purposes (Penos, 1983).
Medicinal plants have been used throughout history,
and efficacy often is associated with anecdotal
folklore. Further estimations suggest that 74% of
119 plant derived drugs were discovered as a result
of chemical studies to isolate the active components
responsible for their traditional use (Farnsworth et
al., 1985). At least 25% of recent medicinal pre-
scriptions contain one active compound from plant
species (Haq, 2000), and the estimated value of
these prescriptions containing plant-derived com-
pounds is worth about $10 billion dollars in the
U.S. alone (Duke, 1990).

The antimicrobial activity of berberine
has been reported. Berberine, isolated from the
Chinese medicinal plant, *Coptidis rhizoma*, demon-
strated antimicrobial activity against seven peri-
odontal pathogens (Hu et al., 2000). The investi-

gators also proposed that a clinical application for
berberine in the treatment of periodontal diseases
might exist. Scazzocchio et al., (2001) examined
the antibacterial activity of β-hydrastine, can-
adine, and canadaline, isolated from *H. canadensis*,
against *S. aureus*, *E. coli*, *P. aeruginosa*, and *S.
sanguis*. They reported β-hydrastine was inactive
against all the tested organisms, but that canadal-
ine and canadine were antimicrobial at comparable
concentrations to berberine. The reduced antimicro-
bial efficacy of the crude extract against the test
organisms therefore may be attributed to the pres-
ence of these other alkaloids and other components.

H. canadensis is considered a rare plant.
The present invention highlights the importance of
identifying the major antimicrobial contributions of
alkaloids from plants, and illustrates that use of
extract components in combination with standard
antibiotics provides a synergistic therapeutic
effect.

The preferred growth mode for many species
of bacteria is biofilm. Biofilm formation takes
place when bacteria attach to a surface from a
liquid phase, producing exopolymeric substances that
aid in the continued attachment to the surface and
offer protection from the external environment.
Bacteria in the form of biofilms have been reported
to be up to 1000 times more resistant to treatment
than the bacteria present in liquid phase (Lewis,
2001; Costerton et al., 1999). This is a serious
problem for the efficacious treatment of infections
(Costerton et al., 1999). Harry et al., (1988)

reported that berberine sulfate at sub-MIC levels inhibited the adherence of *S. pyogenes* to epithelial cells. The results of these tests have shown that berberine and combinations thereof were effective at inhibiting biofilm formation of all the test species except *A. actinomycetemcomitans*.

Because the conventional antimicrobial agents CHX, Tet, and Dox did not affect surface hydrophobicity, beneficial effects could be achieved by combining these antimicrobial agents with berberine. The results from this investigation indicated that berberine was most likely the main contributing agent in reducing cell hydrophobicity. Cell surface hydrophobicity is an important factor in bacterial attachment to a surface and subsequent biofilm formation (Merritt et al., 2000). It also has been proposed that the hydrophobic effect may be the primary driving force for the adhesion of most pathogens (Oliviera et al., 2001). Oral pathogens that have lost their surface hydrophobicity demonstrated significantly less colonization than the parent strains (Matsumoto et al., 1999). Reducing the attachment capability of oral bacteria and other pathogens would be highly desirable, particularly in those persons with recalcitrant infections.

A. actinomycetemcomitans, a facultative anaerobe and potent periodontal pathogen is known for its resistance to antimicrobial treatment, particularly tetracycline antibiotics (Fives-Taylor et al., 2000). Reversal of resistance by synergy produced as a result of the combination of conventional antibiotics and nonantibiotics has been achieved

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(Kristiansen et al., 1997). Although the results from this investigation have shown that *A. actinomycetemcomitans* was not susceptible to this antimicrobial regime, it may be possible to achieve an antimicrobial response if the concentration of agents was increased.

Preliminary investigations have shown that when *A. actinomycetemcomitans* was incubated under anaerobic conditions, it was more susceptible to treatment, demonstrating similar susceptibility profiles as *P. aeruginosa*. The results from this investigation demonstrated that an active efflux is involved in *A. actinomycetemcomitans* resistance to berberine. In addition, the increase in hydrophobicity observed when *A. actinomycetemcomitans* was exposed to the antimicrobial agents demonstrates this organism's wide-ranging ability to resist antimicrobial treatment and highlights the need for efficacious treatment.

P. aeruginosa increasingly is associated with nosocomial infections both in the U.S. and Europe, particularly pneumonia, and can be fatal for immunocompromised persons. *Pseudomonas* infections can be spread within hospitals by healthcare workers, medical equipment, sinks, disinfectant solutions, and food. The bacterium also causes serious problems in individuals suffering from cystic fibrosis, a fatal genetic disorder.

Antibiotic resistance in *Pseudomonas* infections is prevalent. The lack of novel agents in development calls for a need to reexamine the role of colistin therapy in persons with cystic fibrosis

(Beringer, 2001). Colistin is a cationic antibiotic belonging to the polymyxin group. Colistin initially was introduced in the early 1960s, but became redundant by the early 1970s due to reports of its severe toxicity. In this present study, this strain of *P. aeruginosa* was resistant to berberine at the test concentrations, although impairment of growth was observed when combined with CHX and Col. As with the other antibiotics, Tet and Dox, adverse side effects associated with Col can be reduced when combined with berberine. Other strains of *P. aeruginosa* may demonstrate greater susceptibility and, therefore, the beneficial effects of combining Col with berberine cannot be disregarded.

CHX is a widely used biocide in antiseptic and disinfectant products, particularly in hospitals. It has broad spectrum efficacy and is much less irritating to tissue than other products (McDonnell et al., 1999). Chlorhexidine is bactericidal and fungicidal to a wide variety of organisms and it is possible that by combining CHX with berberine, that its activity spectrum may be increased. Bacterial resistance to CHX has been reported, specifically *S. aureus* and *S. epidermidis*, and there is increasing concern regarding the link between biocide exposure and antibiotic resistance (Russell et al., 2000; Russell, 2000; McDonnell et al., 1999; Russell et al., 1996). Combinations of CHX with a natural antimicrobial agent, such as berberine, may result in a more environmentally acceptable product and may serve to increase the

longevity of use and efficacious treatment of oral infections and disinfectant procedures.

Because berberrubine is structurally related to berberine, the results from this investigation suggest that analogs of berberine are antimicrobially active. Synthesized derivatives of protoberberines have been tested against several *Candida* species (Park et al., 2001). The authors also proposed that semisynthetic protoberberines may have considerable potential as novel antifungal agents that lack host toxicity.

A report by Stermitz et al. (1999) hypothesized that plant species containing berberine may evolve multidrug resistance (MDR) efflux pump inhibitors to potentiate berberine activity against pathogens. Resistance to CHX and tetracycline antibiotics theoretically is related to the presence of MDR efflux pumps in bacterial cell membranes which actively remove the agent from the cell. Protoberberine is an amphiphilic cation, similar to CHX, and demonstrates antimicrobial activities, but also is susceptible to extrusion from bacterial cells by MDR pumps (Stermitz et al., 1999). *E. coli* has seven different known MDR pump systems that can export structurally unrelated antibiotics (Sulavik et al., 2001). MDR systems have also been identified in *S. aureus* (Markham et al., 1999), *P. aeruginosa* (Li et al., 1995), and *A. actinomycetemcomitans* (Fives-Taylor et al., 2000).

It is theorized, but not relied upon herein, that the observed synergistic/additive response is related to the inhibition of MDR systems present

in the cell membrane. It also is theorized that
when berberine is used in combination with an anti-
biotic, cells are saturated and are rendered incap-
able of removing the agents simultaneously.

In addition, MDR pump systems are usually
ATP dependent. The increased susceptibility of the
anaerobic species to berberine and combinations
thereof may be related to the reduced amounts of ATP
produced under anaerobic conditions (Prescot et al.,
1999).

It is further proposed that when berberine
is used in combination with other antimicrobial
agents, which are substrates for MDR systems, that
the same additive/synergistic effect could be
achieved. It is possible to achieve the same effect
with non-MDR substrates, because many bacteria
possess different MDR systems.

A report by Sheng et al. (1997) reported
that when berberine was used in combination with
pyrimethamine, good results for treating patients
with chloroquine-resistant malaria was observed.
Another plant alkaloid, reserpine, has been shown to
inhibit the efflux of the fluoroquinolone antibiotic
norfloxacin in wild type *S. aureus* by at least four-
fold (Markham et al., 1999). However, the authors
also proposed that reserpine could not be used to
potentiate the activities of fluoroquinolones be-
cause of neurotoxicity to humans at the concentra-
tions required for efflux inhibition.

If administered in sufficiently high
doses, a goldenseal alkaloid can produce toxic
symptoms ranging from mouth irritation, nausea,

vomiting, and diarrhea to cardiac depression and
central nervous system paralysis (Tice, 1997). It
also has been reported that berberine (100 mg) re-
duced the efficacy of tetracycline in people with
cholera by reducing tetracycline absorption (Khin-
Maung et al., 1985). However, in another report the
findings were inconclusive as to whether berberine
significantly interacted tetracycline antibiotics
(Raddani et al., 1987).

At present, CHX is the "gold standard" of
all plaque control agents and is included in oral
hygiene products. However, CHX is a prescription
drug in the US. In addition, CHX has many side
effects, including undesirable taste, tooth dis-
coloration, and increased calculus (tartar) forma-
tion in users, although no permanent retention in
the body is observed (International Health Care
Foundation, 2001). Tetracyclines are conventional
antibiotics and are routinely used in the treatment
of periodontal disease because of their unique
ability to accumulate in the pocket area. However,
like many conventional antibiotics, many bacteria,
especially important human pathogens, have developed
a resistance to tetracyclines. Tetracyclines also
have many side effects including, gastrointestinal
discomfort, nausea, vomiting, diarrhea, and perma-
nent tooth discoloration in children up to 8 years
old.

Berberine is found in many health supple-
ments in amounts exceeding 50 mg. Antibiotics used
in periodontal treatment include tetracycline and
doxycycline, and are administered typically at 100

mg (doxycycline) to 500 mg (tetracycline) with con-
centrations in the gingival crevicular fluid ranging
from 1 to 8 μg ml^{-1} (doxycycline) and 2 to 12 μg ml^{-1}
(tetracycline) (Slots and Rams, 1990). The present
invention shows that when berberine is administered
in combination with an antibiotic, like CHX, tetra-
cycline, or doxycycline, approximately half to one
quarter the amount of a berberine and an antibiotic
is required to achieve the desired growth inhibition
compared to when either agent is used alone. Syn-
ergy observed *in vitro* also is evident *in vivo*, and
can lead to the development of drug combinations for
the management of infections that are difficult to
treat with one antibiotic alone (Kristiansen and
Amaral, 1997). If a combination treatment using
berberine and a conventional antimicrobial agent is
employed, decreased concentrations of both agents
can be used, thereby reducing the occurrence and/or
severity of adverse side effects.

        Dental plaque frequently is associated
with oral diseases including dental caries and peri-
odontal (gum) disease. Plaque control is of utmost
importance for the prevention of these diseases.
Plaque control can be achieved by mechanical (e.g.,
brushing or flossing) and chemical means. For the
latter, antimicrobial agents have been incorporated
into oral hygiene products, such as toothpastes and
mouth rinses, to control plaque and gingivitis/-
periodontal disease. Therefore, a composition con-
taining reduced levels of CHX, tetracycline, doxy-
cycline, or other antibiotics, that minimizes
occurrence or severity of the above-mentioned ad-

verse effects while achieving the desired antimicro-
bial effect would be a significant advance in the
art.

The efficacy of the combination treatment
against two well known human pathogens, *E. coli* and
S. aureus, also was demonstrated *in vitro*. The re-
sults indicate that when berberine chloride is used
in combination with CHX or a tetracycline antibiotic
(including tetracycline hydrochloride and doxy-
cycline hydrochloride), a synergistic growth inhibi-
tion effect is observed. These findings suggest
that the present combination treatment can provide
new therapeutic agents for treatment of oral and
other diseases.

In summary, the above results demonstrate
potentiative interactions between an antibiotic and
a berberine in a novel management of oral and non-
oral infections.

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